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A Preliminary Chromatographic Study of Eastern American Dryopteris

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The use of chemical methods—especially serology, electrophoresis, and chromatography—is fairly new in plant taxonomy. In fact, our knowledge of chemotaxonomy is still so far in its embryonic stages that probably the significance of chemical data is not at all understood. The present investigation was made to determine what chromatographic results could be obtained from studying eastern American woodferns, *Dryopteris*. These ferns are among the best known in the United States cytogenetically, thanks to the work of Walker (1955, 1959, 1961, 1962) and are accordingly an ideal group to examine, especially because numerous hybrids are known. The work focused not only upon the species, but the interspecific hybrids as well.

The chromatographic method makes it possible to take extracts from different kinds of plants and to compare the individual compounds present. It has been found that species, varieties, and hybrids of plants and animals often show strikingly different chromatographic patterns (Alston and Turner, 1963).

The application of chromatography to flowering plants has been fairly widespread during the past few years, but such study of ferns has been slight. Recently Smith and Levin (1963) investigated the Appalachian *Aspleniums* chromatographically and found striking evidence in support of a theory of reticulate

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evolution for these plants. By the use of chromatography Fikenscher and Gibson (1962) compared phloroglucinol derivatives among the woodferns *Dryopteris intermedia*, *D. spinulosa*, *D. intermedia* \times *spinulosa*, *D. clintoniana*, *D. clintoniana* \times *goldiana*, *D. arguta*, *D. goldiana*, and *D. marginalis*. They concluded that the hybrids showed additive phenomena of the compounds present in the parent species. This kind of result did not prove to be the case in the present study, as will be described below.

MATERIALS AND METHODS

Plants of various species and hybrids were grown under essentially uniform conditions in Birmingham, Michigan, by Hagenah of the Cranbrook Institute of Science. On July 30, 1963, comparable leaves, all with sori present, were collected from each plant. Vouchers for each plant are deposited in the University of Michigan Herbarium. Leaf material of *D. goldiana* was taken from a natural habitat near Ann Arbor on July 31 and that of *D. clintoniana* \times *spinulosa* from the University of Michigan Botanical Gardens on the same date; all others were provided from the Hagenah garden. Because of their special interest, the *D. spinulosa* and the *D. cristata* complexes were studied in two dimensions. (These and other hybrids were examined in one dimensional chromatograms also but will not be reported here.)

Mature, healthy fronds were cut finely and extracted for 30 hours in 1 percent methanolic 1N HCl. These extracts were condensed and 60 λ spotted on 46 \times 57 cm. sheets of Whatman no. 1 filter paper. The two-dimensional descending method was employed, using N-butanol: acetic acid: water (at volume/volume ratio of 4:1:2) for the first separation of 20 hours and 1 percent HCl for the second dimension of four hours, both at $21^{\circ} \pm 1^{\circ} \text{C}$. The solvents were mixed six hours prior to use. Equilibration of the chromatographic chamber lasted five hours in both cases. All equipment and solvents were stored two days prior to use at the same temperature. The chromatograms were dried and

inspected for position of the solvent fronts and position and intensity of compounds under short and long wave ultra-violet light. Of several chromogenic sprays tested, 2N KOH gave the best results and was used throughout. The chromatographic patterns were copied before and after spraying with differently colored wax pencils on sheets of clear acetate (Scora, 1964). These acetate sheets were then superimposed to show pattern deviations.

RESULTS

The smallest number of spots in the chromatograms was found in *Dryopteris campyloptera* \times *intermedia*, and the largest number of chemical substances in *D. dilatata* \times *intermedia* and in *D. clintoniana* \times *goldiana*—a range from 6 to 16.

The effects of hybridization were striking, and all of the following situations were observed in our chromatograms:

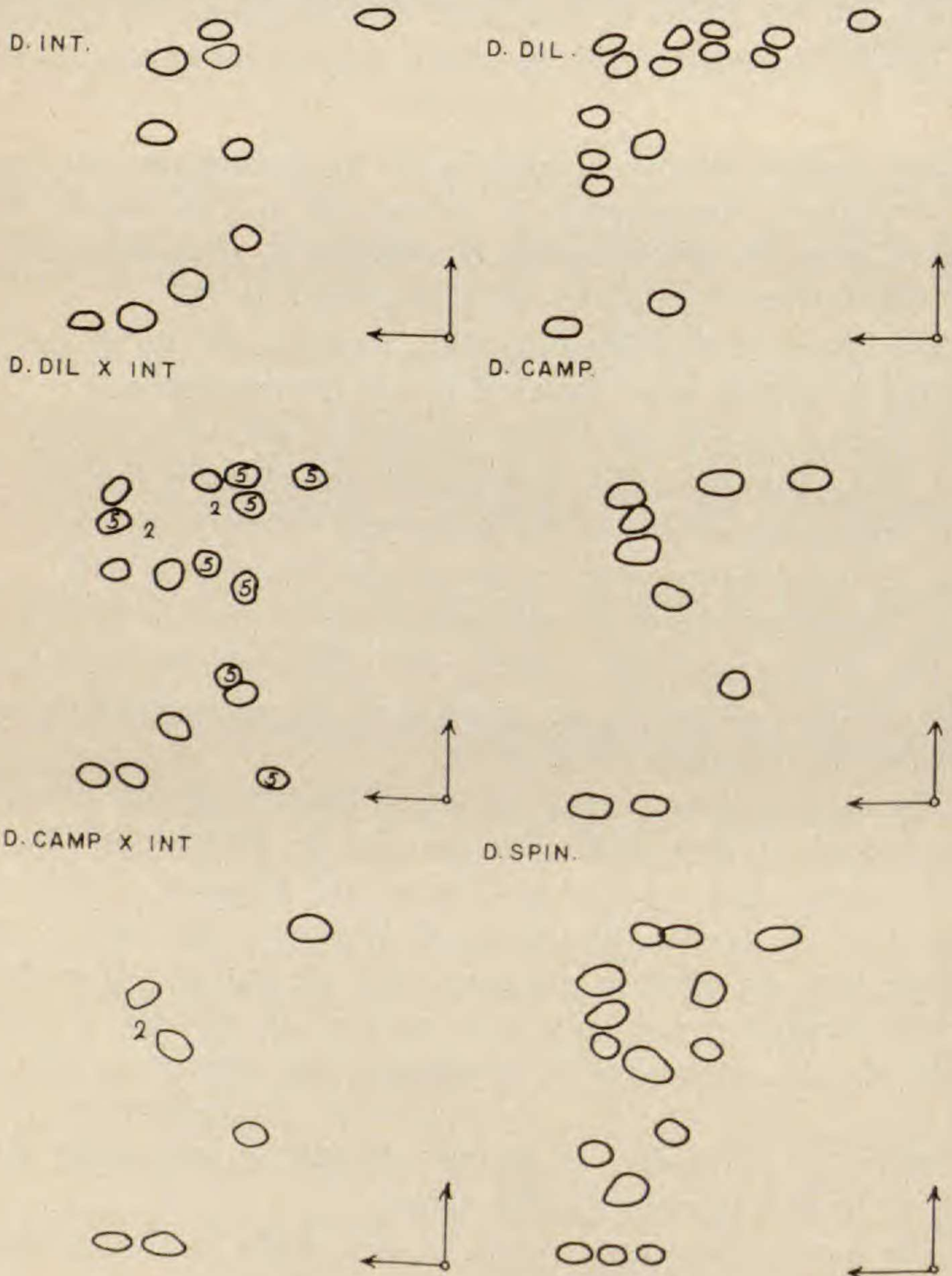
1. Substance *present in both parents* and *present* in hybrid.
2. Substance *present in both parents* and *absent* in hybrid.
3. Substance *present in one parent* and *present* in hybrid.
4. Substance *present in one parent* and *absent* in hybrid.
5. Substance *absent in both parents* and *present* in hybrid.

The chromatographic entities that illustrate the second and fifth of these situations are marked by the corresponding numbers on the diagrams (Figs. 1-3).

The most striking chromatogram obtained is the one involving the hybrid of *Dryopteris dilatata* and *D. intermedia* (fig. 1). This hybrid, first reported by Wagner and Hagenah (1962), was found in the Huron Mountains of Michigan, and subsequently grown from an offset in company with the parents. One-dimensional chromatograms only were made of *D. dilatata* \times *marginalis* and *D. intermedia* \times *marginalis*, but these were different in nature from similar one-dimensional chromatograms of *D. dilatata* \times *intermedia*. *Dryopteris dilatata* \times *intermedia* shows all of the five situations listed above.

The most conspicuous result of this study is that wherever

taxa of different polyploid levels are involved, the hybrid chromatograms usually tend to have more substances in common with the parent of higher ploidal level than with the one of lower level. This is shown by *Dryopteris campyloptera* (4X) × *intermedia* (2X); *D. intermedia* (2X) × *spinulosa* (4X)—(fig. 2); and *D. cristata* (4X) × *intermedia* (2X)—(fig. 2).



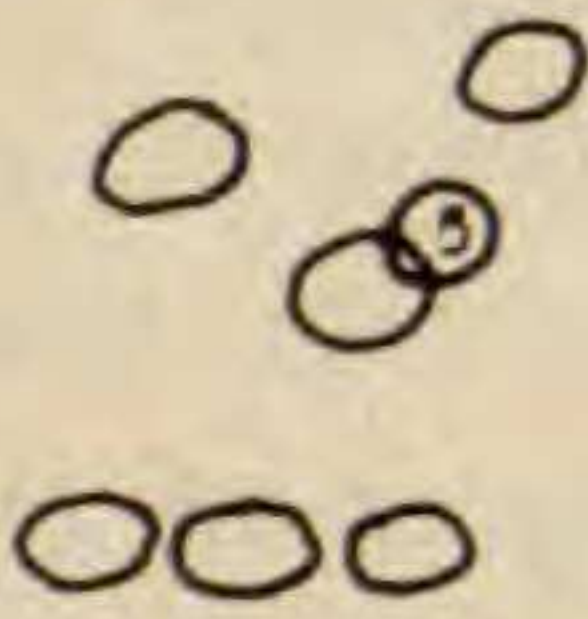
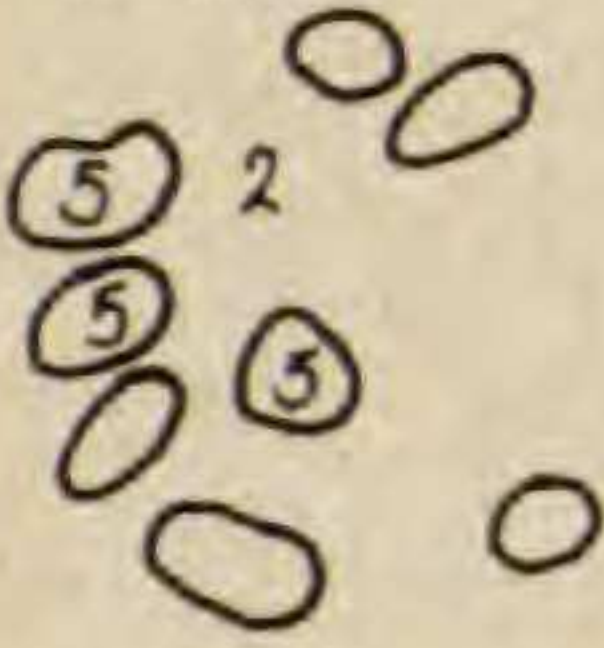
The one exception to this pattern is an unusual hybrid involving *Dryopteris clintoniana*, a hexaploid, and *D. spinulosa*, a tetraploid (fig. 3). In this plant the hybrid is unusual in showing more resemblance to the lower polyploid than to the higher. However, as has been found by Walker (1955, 1962) at least half of the genetic influence of *D. spinulosa* is probably already present as one of the three genomes of *D. clintoniana* (Walker, 1955, 1962). Thus the composition of *D. clintoniana* \times *spinulosa* cannot be represented by the formula ABC plus DE, but rather ABC plus CD. Perhaps this genome homology between *D. spinulosa* and *D. clintoniana* is responsible for this turnabout in the pattern previously indicated (namely that the higher polyploid tends to have more influence than the lower).

DISCUSSION

It is clear from the results obtained in this study of members of the *Dryopteris spinulosa* and *D. cristata* groups that (1) fairly clear-cut differences exist between the species—as has been suggested by the previous work of Fikenscher and Gibson; and (2) interspecific hybrids by no means show clear-cut blending of parental patterns. The hybrid patterns are neither precisely intermediate, nor are they shown to be additive by the methods we used. If such a study as the present one had been made on only two parental species and a suspected hybrid, the worker might have concluded that the suspected hybrid was not a hybrid on the basis of the chromatic patterns even though it

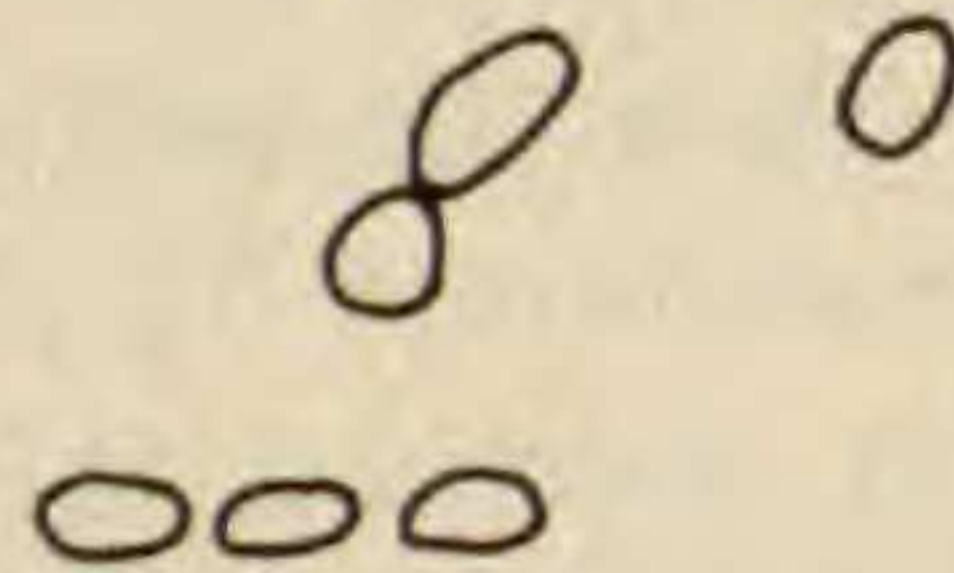
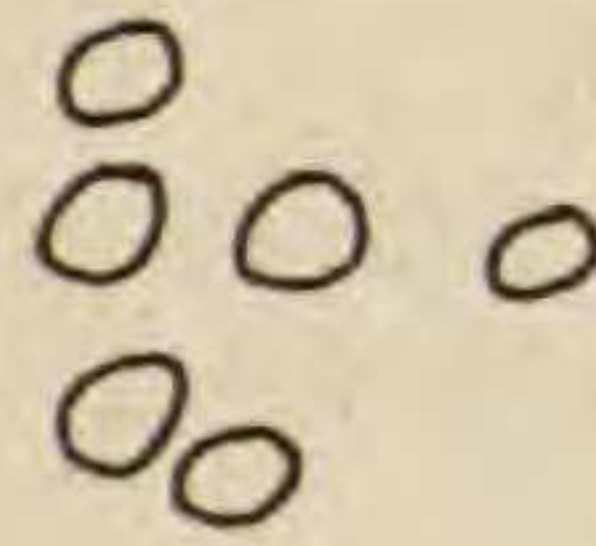
FIGURE 1. TWO DIMENSIONAL CHROMATOGRAMS OF *Dryopteris intermedia*, *D. dilatata*, *D. dilatata* \times *intermedia*, *D. campyloptera*, *D. campyloptera* \times *intermedia* AND *D. spinulosa*, AS SEEN IN LONG WAVE U/V LIGHT AFTER TREATMENT WITH 2N KOH. THE NUMBER 2 DENOTES POSITION OF SUBSTANCES PRESENT IN BOTH PARENTS AND ABSENT IN HYBRID. NUMBER 5 DENOTES "HYBRID CHARACTERISTIC SUBSTANCE" ABSENT IN BOTH PARENTS. HORIZONTAL ARROW INDICATES DIRECTION OF FIRST SEPARATION WITH N-BUTANOL: ACETIC ACID: WATER. ASCENDING ARROW INDICATES DIRECTION OF SECOND SEPARATION INVOLVING 1% HCL.

D. INT X SPIN

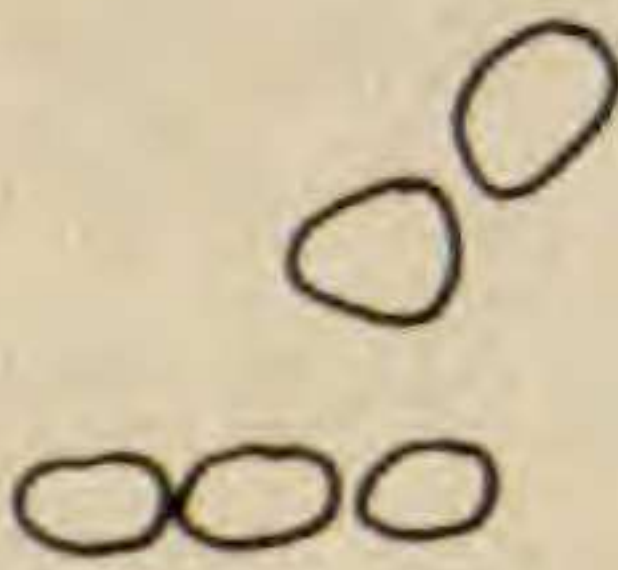
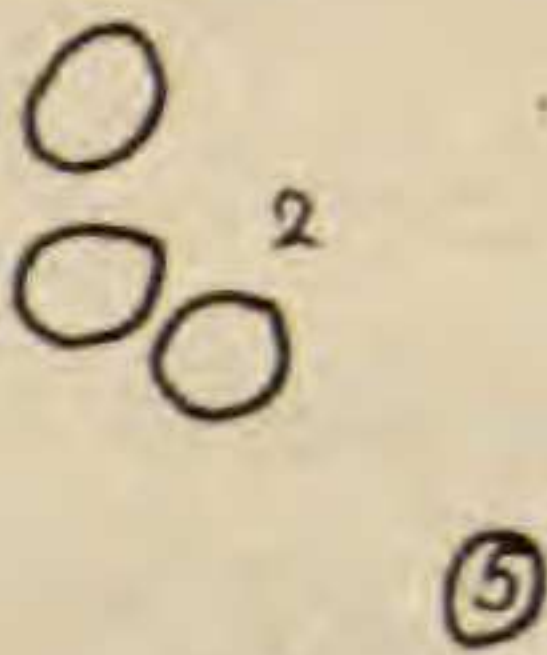


D. CRIST X INT

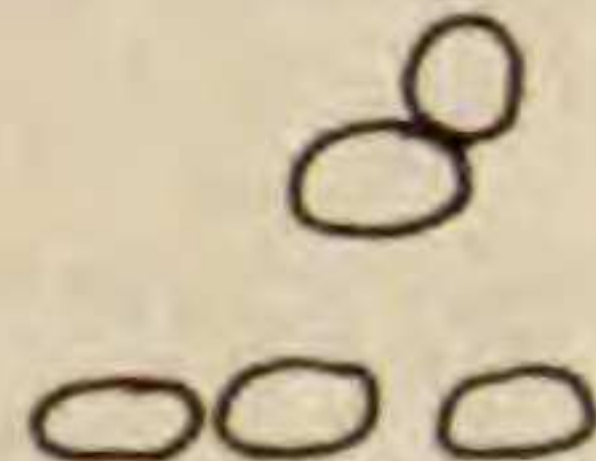
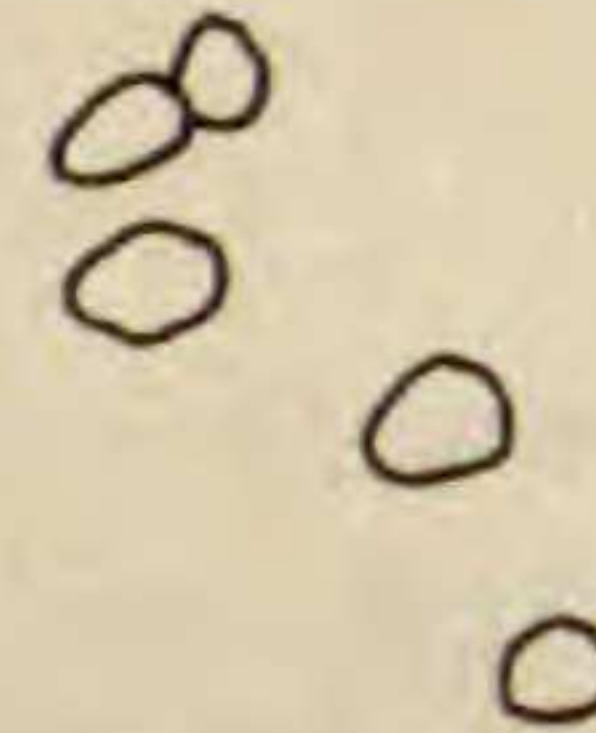
D. CRIST



D. GOLD.



D. CRIST X GOLD
D. CLINT.



D. CLINT X GOLD

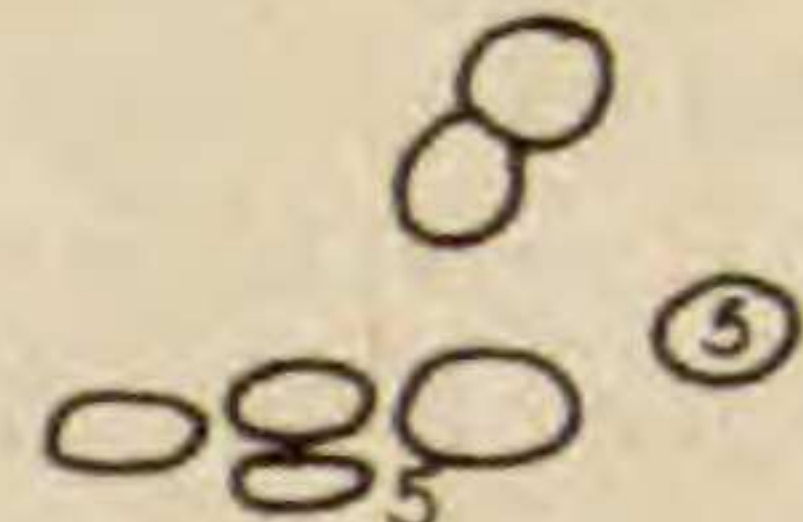
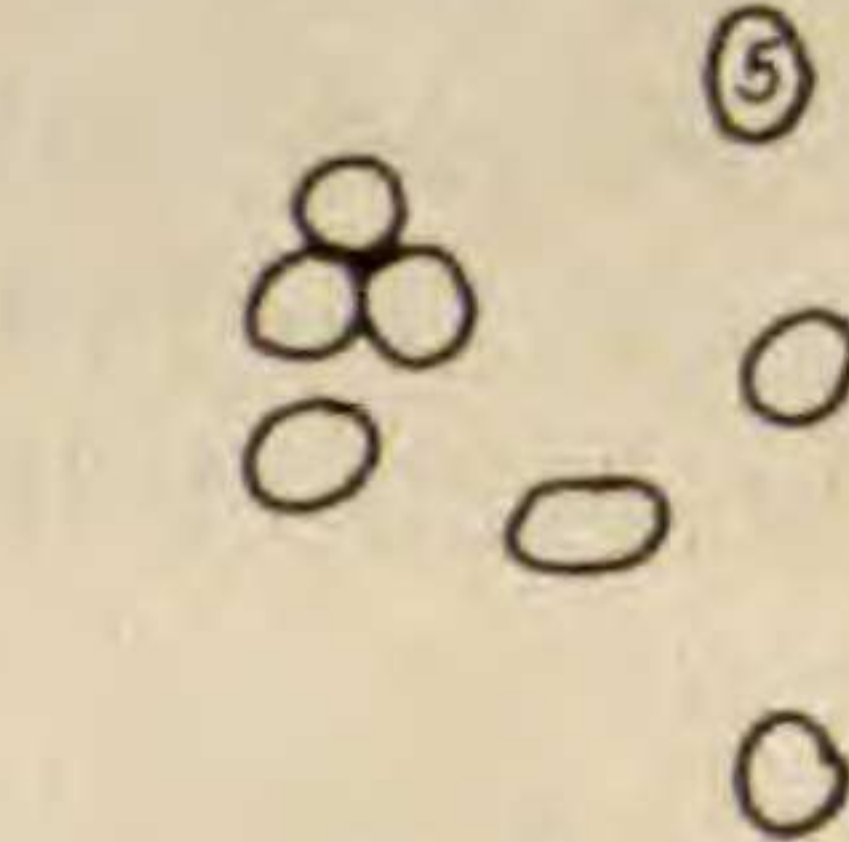
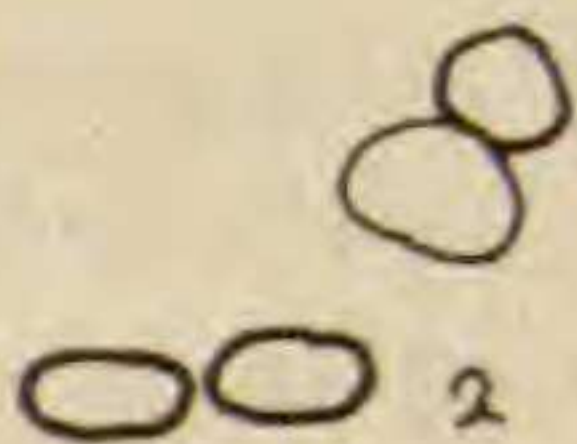
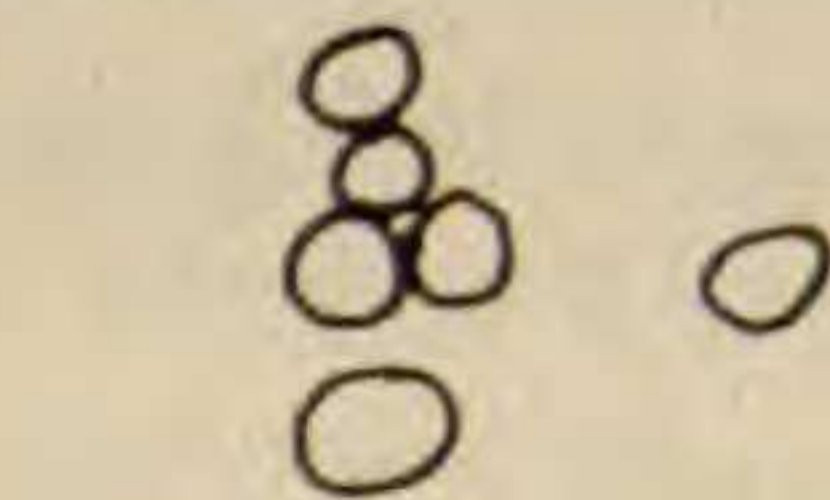


FIGURE 2. TWO DIMENSIONAL CHROMATOGRAM OF *Dryopteris intermedia* X *spinulosa*, *D. cristata*, *D. cristata* X *intermedia*, *D. goldiana*, *D. cristata* X *goldiana*, *D. clintoniana*, *D. clintoniana* X *goldiana*. OTHERWISE AS FIGURE 1.

were intermediate morphologically. A method like the present one, if used without reference to other data, might lead to serious errors. In the present instance a great deal was known about the plants involved through morphological and cytogenetic studies, and the chances were extremely small that the plants considered hybrids were not hybrids. The bulk of the specimens had been examined cytologically and found to have pairing behavior that confirms their hybrid origin; further, their morphology is intermediate between the suspected parents.

This research was carried out on single plants of each of the taxa involved. No test was made of whether or not each substance found in the basic species was wholly "species-characteristic" and there is no guarantee that the same chromatograms would be obtained with other biotypes or other clones of the species. If one were to argue that perhaps the chromatographic averages of many of the same hybrid combinations would be intermediate between the "averages" of the parents, then such data, as shown by the results here, at least, would be individually so variable as to be utterly useless as taxonomic criteria. However, the fact that we made two-dimensional chromatograms of eight hybrid combinations involving six different sexual species, and obtained essentially the same tendencies in practically all of

D. CLINT X INT

D. CLINT X SPIN

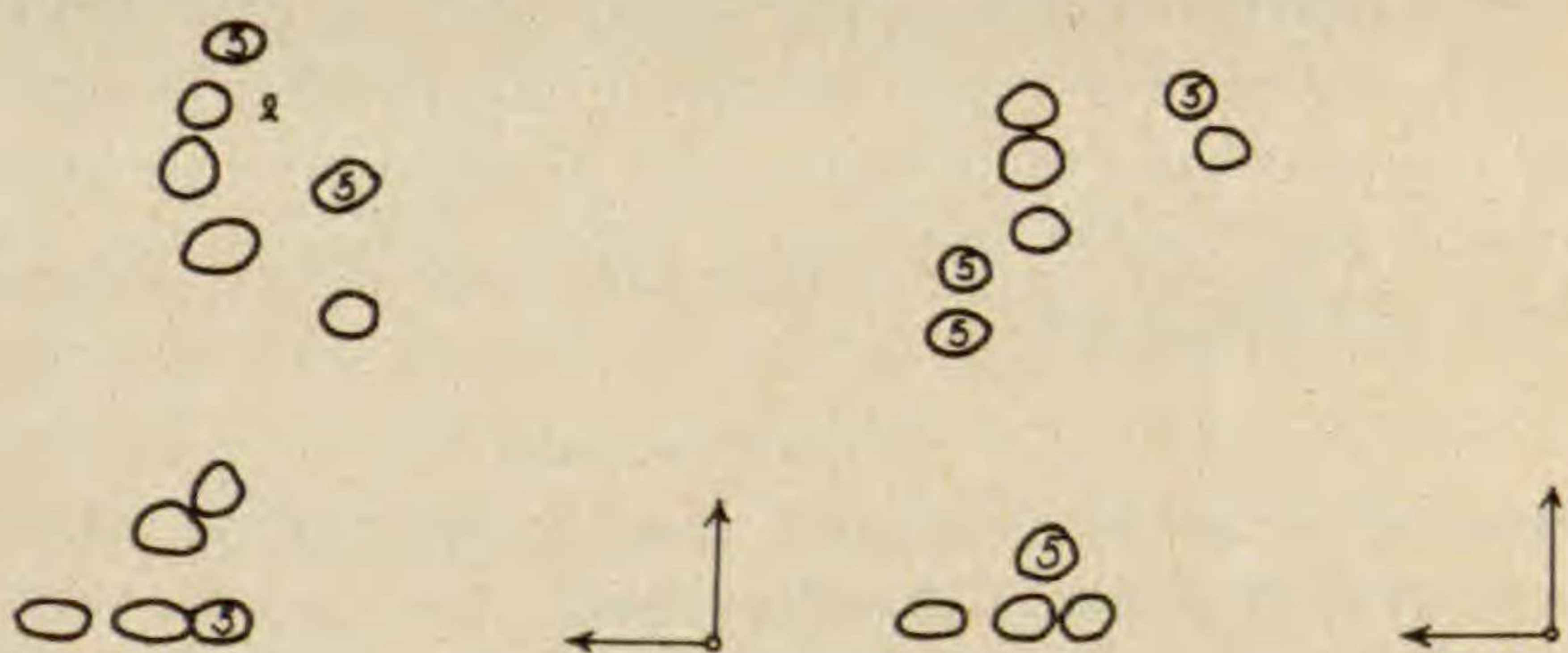


FIGURE 3. TWO DIMENSIONAL CHROMATOGRAM OF *Dryopteris clintoniana* \times *intermedia* AND *D. clintoniana* \times *spinulosa*. OTHERWISE AS FIGURE 1.

them (the sole exception being *D. clintoniana* \times *spinulosa*, as explained above) indicates that the basic conclusions drawn from this work probably are reliable.

Why do the hybrids not show neatly intermediate or additive patterns of chemical compounds? Compounds which are present in one or both parents seem to disappear completely in the chromatograms of the hybrids. Or, even more surprisingly, wholly new spots apparently representing new substances not present in the parents, may appear. All this indicates that it is apparent that the biochemical reactions that lead to the formation of characteristic substances in the various woodferns differ from species to species. When hybridization occurs, the new biotypes may have "hybrid" reactions. These may take different forms, and unexpected changes may appear in the hybrids. For example, a dominant gene from one parent could prevent a reaction controlled by a recessive gene of the other parent from occurring, or the interaction of two different genetic systems controlling reactions may actually produce a substance different from any present in the parents, and a new spot might accordingly appear on the chromatogram. One disturbing possibility is that a very slight difference in a synthetic process such as adding or losing a reactive group might make a radical difference in the chromatographic behavior of a substance. Therefore, chromatograms alone may not always give tangible evidence of whether or not a particular plant is of hybrid origin. Indeed, in some taxonomic groups it will perhaps become necessary to understand the genetics and detailed biochemistry of the parental and hybrid substances before patterns can be interpreted and evaluated.

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Ferns Associated With Ultramafic Rocks in the Pacific Northwest¹

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The unique plant life on serpentine and other ultramafic rock types is a telling reminder to plant ecologists, taxonomists, and plant geographers of the significance of the edaphic factor in plant distribution. Endemism, ecotypic differentiation, serpentinomorphism, singular vegetational physiognomies, and "extralimital" distributions all contrive to make the floras of these magnesium-rich, calcium-poor areas fascinating and unique botanical areas (Krause, 1958; Whittaker, 1954). During the course of genecological and floristic studies on the plant life of ultramafic outcrops in the Pacific Northwest,¹ I have been struck by the highly predictable recurrence of and restriction to ultramafic soils of three fern species. *Polystichum mohrioides* (Bory) Presl var. *lemmonii* (Underw.) Fern. and *Cheilanthes siliquosa* Maxon

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